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Nitrogen Removal by Sulfur-Based Carriers: Effect of Physical Properties of Carriers on Autotrophic Denitrification

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Abstract

Sulfur-based autotrophic denitrification is an effective treatment technique to remove nitrogen in water and wastewater, in which sulfur compounds act as an electron-donors for the conversion of nitrate to nitrogen gas by autotrophic microorganisms. The main advantage of this process is no external organic carbon source compared with the conventional heterotrophic denitrification process, which resulted in reducing the cost of treatment and the poisoning effect of some organic carbon compounds. In this study, two sulfur-based carriers (C1 and C2) which have different mass ratios with the same core components as elemental sulfur and calcium carbonate but varying adhesive volumes were prepared to evaluate the sulfur-driven denitrification performance. The results show that the nitrate removal rates of C1 and C2 were 0.34 ± 0.04 and 0.32 ± 0.02 kg NO_3^--N/m^3/d, respectively. Beside that, the sulfate concentration generated by autotrophic denitrification were quite high at 273.5 ± 27.8 and 251.3 ± 17.0 mg SO_4^{2-}/L, respectively.

Keywords: Sulfur-based carrier, autotrophic denitrification, pore size, thiobacillus, sulfurimonas.

1. Introduction

Vietnam is a developing country, so agriculture plays an important role in the nation's economy. Livestock farming is strongly developing in the agriculture field, however, wastewater generated from the farms becomes a worrying problem. The main components of this wastewater are organic and nutrient compounds.

Nitrate may cause negative impacts on human health such as methemoglobinemia (too little oxygen delivered to cells), blue baby syndrome (baby born with blue or purple color skin), cancer disease in the gastrointestinal tract; on the aquatic environment such as ecological imbalance and eutrophication. Moreover, the reinforcement of the standard for effluent and the reuse of treated water have recently been encouraged due to the increase in water pollution and depletion of water. Various solutions have been suggested for nitrate treatment such as electrodialysis, reverse osmosis, adsorption, distillation, ion exchange (physicochemical processes), autotrophic denitrification, heterotrophic denitrification, mixotrophic denitrification (biological processes). Nevertheless, the physicochemical processes have several drawbacks such as high treatment costs, generation of brine wastes (e.g., distillation membrane, reverse osmosis), and low selectivity (e.g., ion exchange, adsorption). Therefore, biological methods have been attended for nitrogen removal because of low treatment cost, and lower energy consumption.

It is required to improve the existing biological treatment process to efficiently treat nitrogen in the water system. In general, biological treatment processes such as nitrification by autotrophic microorganisms and denitrification by heterotrophic microorganisms are mainly used for nitrogen removal. Heterotrophic denitrification has been widely applied for nitrate removal due to its inherently high denitrification rate, however, this process entails adequate organic carbon for complete denitrification occurrence. It is a challenge for wastewater containing a low C/N (carbon-to-nitrogen) ratio such as leachates, agriculture wastewaters, and livestock farming effluents [1, 2]. In this case, an external organic carbon source will be supplemented, resulting in a secondary pollutant by residual organic compounds.

To overcome this drawback, autotrophic denitrification is a potential alternative in which sulfur acts as an electron donor for the conversion of nitrate to nitrogen gas by autotrophic bacteria (e.g., *Thiobacillus*, *Sulfurimonas*) [3,4]. The sulfur-driven autotrophic denitrification is expressed in the below equation, in which nitrate and elemental sulfur compounds serve as electron acceptors and donors.

$$1.10S^o + NO_3^- + 0.40CO_2 + 0.76H_2O + 0.08NH_4^+ \rightarrow 1.10SO_4^{2-} + 0.5N_2 + 0.08C_5H_7O_2N + 1.28H^+$$

Autotrophic denitrification processes have been currently divided into two groups: hydrogen-based, in which hydrogen gas is used, and sulfur-based, in which sulfur compounds, such as sulfide and elemental

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sulfur, are used as electron donors. Because of its clean and harmless advantages, hydrogenotrophic denitrification has gotten a lot of interest in recent years. The biggest challenge, though, is obtaining hydrogen. Since hydrogen is volatile and explosive, it can be dangerous to transport and store. As a result, supplying hydrogen directly to the reactor is not a viable option. Furthermore, producing hydrogen in situ via water electrolysis necessitates an additional energy supply, which raises the operational cost. The sulfur-based technique has grown in popularity as a result of the dangers and difficulties associated with handling hydrogen gas.

Autotrophic denitrification has many advantages compared to the heterotrophic denitrification process such as less biomass and N_2O (nitrous oxide - a greenhouse gas) production, resulting in lower sludge treatment costs; no organic carbon supplementation, resulting in lower operating costs and the limitation of residual organic carbon effects. However, this process has also several disadvantages such as sulfate generation, alkalinity consumption, and long-term incubation time. Among sulfur compounds (e.g., S^{2-} (sulfide), S^0 (elemental sulfur), and $S_2O_3^{2-}$ (thiosulfate)) used for autotrophic denitrification, elemental sulfur has been widely used due to its characteristics of inexpensive, available, non-poisonous, easy to transport, and no inhibition on the denitrification process. Additionally, when elemental sulfur is used, the amount of sulfate generation throughout the process is limited. While elemental sulfur appears to be a potential substrate in denitrification, its poor solubility may severely limit its availability to microorganisms, introducing an inherent weakness into this process.

However, most previous studies have utilized elemental sulfur particles, which cause several problems in operation such as channeling, clogging, and fouling [5]. Thus, a new type of carrier was fabricated in this study from elemental sulfur and calcium carbonate which are called sulfur-based carriers. When operated under appropriate conditions, the nitrate removal rate can be achieved at around 0.36 kg NO_3^--N/m^3/d [6].

On the other hand, the physical properties of carriers such as pore size and porosity have a strong effect on the sulfur-driven autotrophic denitrification performance, and the bigger pore size would be suitable for autotrophic denitrifiers to penetrate inside [7]. Consequently, sodium silicate or distilled water was added to the composition of carriers, and the efficiency in denitrification improvement of two solutions was evaluated in this study.

2. Materials and Research Method

In this study, two types of sulfur-based carriers (C1 and C2) were fabricated with specific components illustrated in Table 1. Elemental sulfur replaces organic carbon as an electron donor in the denitrification process. Unlike heterotrophic denitrification, the sulfur-driven autotrophic denitrification consumes alkalinity, besides, the autotrophs need inorganic carbon such as bicarbonate, carbonate, and carbon dioxide as carbon sources for microbial metabolisms. Thus, $CaCO_3$ (calcium carbonate) was used to make sulfur-based carriers. Sulfur and calcium carbonate powder was mixed with a mass ratio 5 : 4, then $NaSiO_3$ (sodium silicate) solution known as water glass was added to the mixture to stick components together [6]. In the case of carrier C1, distilled water was added to halve the volume of sodium silicate used. Then, the carriers were shaped with a dimension of 10mm × 10 mm× 10 mm by silicone molds and dried at a temperature of 105 °C in the oven for 6 h.

In the first stage, the seed activated sludge was collected from an aerobic tank of a domestic wastewater treatment plant in Ho Chi Minh City (Vietnam) with an initial MLSS (mixed liquor suspended solids) concentration of approximately 4000 mg/L. The sludge was then enriched by a mineral medium (Table 2) with a volume ratio of 1:9 [8, 9]. The enrichment process was conducted in a sealed batch bioreactor (a working volume of 5 L as shown in Fig. 1a) until the autotrophic denitrifiers were increased. A stirrer was installed near the bottom of the bioreactor with a stirring rate of 20-30 rpm to avoid biomass settling [6].

Table 1. Composition of elemental sulfur-based carriers

		C1	**C2**
Main components	S^0	5.0 g	5.0 g
	$CaCO_3$	4.0 g	4.0 g
Adhesive	$NaSiO_3$	2.5 mL	5.0 mL
	Water	2.5 mL	0 mL

Table 2. Medium and synthetic wastewater used in this study

Composition (mg/L)	Medium for enrichment stage	Synthetic wastewater
KNO_3	560	82
K_2HPO_4	430	6.5
$MgCl_2.6H_2O$	-	9.5
$ZnSO_4.7H_2O$	-	0.2
$MnCl_2.4H_2O$	-	0.05
$FeSO_4.4H_2O$	-	2.0
$NaHCO_3$	220	-
$Na_2S_2O_3.5H_2O$	800	-
$MgSO_4$	110	-

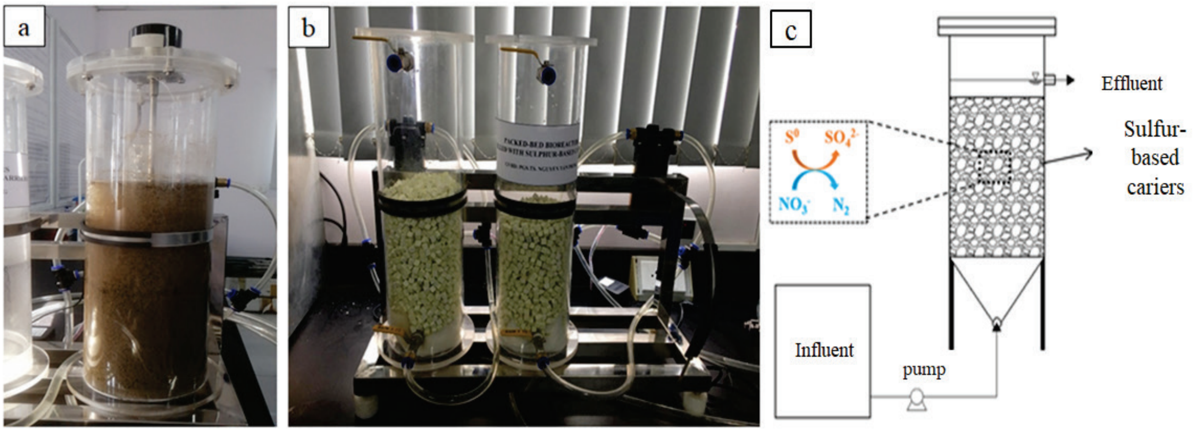

Fig. 1. Lab-scale bioreactors in this study; (a) a sealed batch bioreactor for enrichment; (b) Two packed-bed bioreactors filled with sulfur-based carriers; (c) structural diagram of the packed-bed bioreactor

After enrichment, the biomass was fed into the packed-bed bioreactors. In the second stage, two types of carriers (C1 and C2) were added to two packed-bed bioreactors with a 80% occupancy in these reactors [6]. The packed-bed bioreactors are cylindrical in shape, with a working volume of 5.0 L and a dimension of D × H = 140 mm × 470 mm (Fig. 1b). Feed pumps were set at a flow rate of 1 L/h to supply synthetic wastewater (Table 2) for autotrophic denitrification. The characteristic of inlet were COD of 0 mg/L, NO_3^--N of 52.3 ± 2.1 mg/L, NO_2^--N of 0.002 ± 0.001 mg/L, and SO_4^{2-} of 11.9 ± 1.3 mg/L. The hydraulic retention time (HRT) was controlled at 3 h [6]. Besides, no devices were utilized to adjust the temperature and dissolved oxygen (DO) concentration in this study. During the operation time, the temperature was also measured daily and the value was fluctuated at 25-30 °C. The effluent samples were daily collected to analyze the wastewater quality. Parameters such as pH, nitrite (NO_2^--N), nitrate (NO_3^--N), sulfate (SO_4^{2-}) were determined according to standard methods [10] to evaluate the nitrogen removal performance.

3. Results and Discussion

3.1. Enrichment Stage

Percentage of de-nitrifiers which are involved in nitrate conversion in activated sludge is quite low due to the lack of anoxic conditions in conventional aeration biosystems. Thus, the initially activated sludge was enriched by a suitable mineral medium to shorten the adaptation period and enhance the denitrification efficiency. The mineral medium was daily refilled to maintain the nutrient concentration for autotrophic bacteria. The data in Fig. 2 indicated that the nitrate in the enriched bioreactor was not removed in the first five enriched days. The effluent nitrate concentration was relatively high (41.3 ± 1.7 mg/L) and insignificantly different from the influent. This proves that the percentage of denitrified bacteria in the initial community is quite low. However, from the 6th day onwards, the NO_3^--N concentrations decreased to 24.1 ± 0.3 mg/L. The nitrogen removal efficiency was improved by an average of 69 ± 8% after 13 days, and until the 20th day, the efficiency was achieved at the highest value of 93%. Since there is no organic carbon in the mineral medium, the nitrogen in the bioreactor was removed by autotrophic denitrified bacteria. It is clear that enriched activated sludge can improve nitrogen removal compared to that of conventional activated sludge, after 26 days the process was more stable performance, and the enriched sludge was added to packed-bed bioreactors for the second stage.

In addition, sulfate being one of the final products of sulfur-based autotrophic denitrification was also determined. The influent sulfate concentration was 14.3 ± 2.6 mg/L, whereas the effluent sulfate concentration continuously increased during the enrichment period of 26 days (Fig. 2). The development of sulfate concentration demonstrated that the autotrophic denitrification performance became better over time, and the autotrophic denitrifiers seemed to be dominant in the microbial community.

3.2. Comparison of Nitrogen Removal by Two Types of Sulfur-Based Carriers

Performance of autotrophic denitrification of two different sulfur-based carriers showed in Fig. 3. The influent NO_3^--N concentration was maintained at 52.3 ± 2.1 mg/L, corresponding to a nitrogen loading rate of around 0.42 ± 0.05 kg NO_3^--N/m^3/d for both packed-bed bioreactors. From the 1st day to the 16th day, the bioreactor filled with C1 carriers showed a relatively stable removal performance, and the highest nitrate removal efficiency was obtained at 92 ± 2% on the 16th day, while the bioreactor filled with C2 carriers exhibited a fluctuated removal performance, and the highest nitrate removal efficiency of 84 ± 2% was achieved on the 15th day. From the 18th day onwards, the autotrophic denitrification performance of both bioreactors was more stable. Overall effluent NO_3^--N concentration in the two packed-bed bioreactors were 9.6 ± 1.0 mg NO_3^--N/L and 12.1 ± 0.7 mg NO_3^--N/L, respectively for the carrier C1 and C2. In other words, nitrate removal efficiencies were obtained at 82 ± 8% and 77 ± 5% for carriers C1 and C2, respectively. This phenomenon demonstrated that sulfur-based carriers have capable of stimulating the autotrophic denitrification process to occur in the packed-bed bioreactors filled with sulfur-based

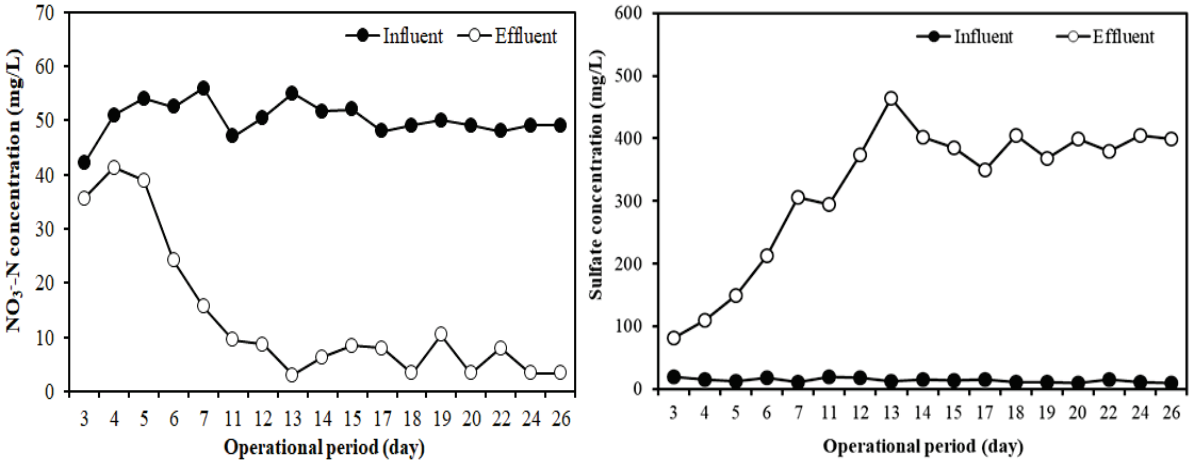

Fig. 2. Variation of nitrate and sulfate concentration during the enrichment phase

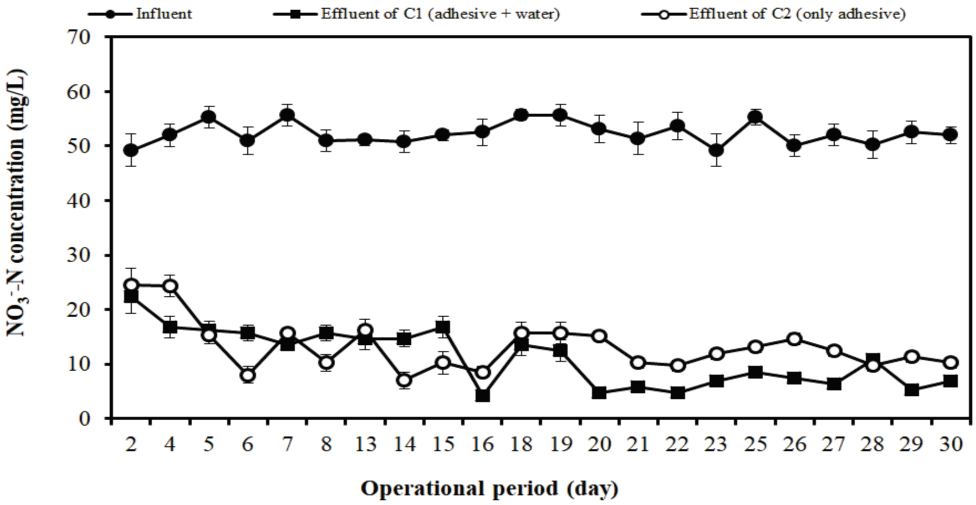

Fig. 3. Variation of nitrate concentration during the main operational phase

carriers. The nitrogen removal rates achieved are also quite high. These carriers should be considered to apply for nitrogen treatment in wastewater, especially for wastewater containing low C/N ratios. The removal mechanism of the sulfur-driven autotrophic denitrification process was illustrated in Fig. 4.

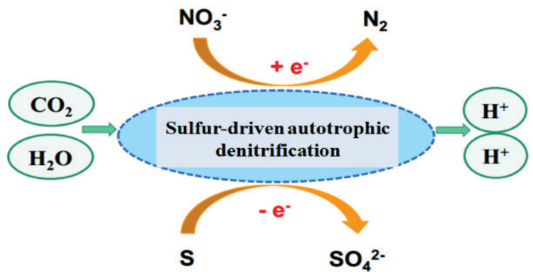

Fig. 4. Removal mechanism of the sulfur-driven autotrophic denitrification

Additionally, the results indicated that the C2 carriers exhibited a slightly lower nitrate removal efficiency than the C1 carriers. This is explained by the increase in the porosity and pore size of C1 carriers due to adding water to the fabrication materials. The surface morphologies of carriers C1 and C2 were analyzed by SEM (Scanning electron microscope). Random samples of C1 and C2 (10 samples for each carrier) were selected and sent to German - Vietnamese Technology Academy (Ho Chi Minh City University of Industry and Trade, Vietnam).

Data indicated that the average porosity and pore size of carrier C2 was 62 ± 2% and 1.41 ± 0.10 µm, respectively. These parameters were improved by adding an additional amount of distilled water in the component instead of solely use sodium silicate with the mixture of sulfur and calcium carbonate. Particularly, the porosity of sulfur-based carriers was enhanced from 62 ± 2% (C2 without water addition) to 69 ± 2% (C1 with water addition), as well as the pore size also was enlarged from 1.41 ± 0.10 µm (C2 without water addition) to 3.17 ± 0.12 µm (C1 with water addition). In addition, the expansion of the pore size can improve the specific area of carriers, leading to the enhancement of biomass concentration attached inside the sulfur-based carriers. However, along with the increase in pore size and porosity, the hardness of the carriers also was decreased. The bulk density of carrier C2 was determined at 0.84 g/cm^3 but decreased at 0.69 g/cm^3 in the case of carrier C1. Thus, ensuring the durability of the material during operational periods should be considered. The other study also demonstrated that by adding amount of water instead of solely using sodium silicate, the porosity enhanced from 60% (without water) to 67% (with water), and the pore size of carriers enhanced from 1.40 µm (without water) to 3.13 µm (with water) [7].

Influent sulfate concentration of both the packed-bed bioreactors was maintained at 11.9 ± 1.3 mg/L, but the effluent sulfate concentrations fluctuated during the operational period. In the first few days, the sulfate concentration generated by autotrophic denitrification was around 150 mg/L then increased in the following days. More sulfate production indicated that more nitrate was removed. The average sulfate concentrations in the effluents of C1 and C2 were measured at 273.5 ± 27.8 and 251.3 ± 17.0 mg/L, respectively (Fig. 5). These values are consistent with the nitrate removal efficiencies of two types of carriers as mentioned above. According to theory, 7.54 mg of SO_4^{2-} is produced when 1 mg of NO_3^--N is removed. The ratios of effluent sulfate to the removed nitrate in the first and second bioreactors were 6.11 and 5.93 mg SO_4^{2-}/mg NO_3^--N, respectively. The actual ratio is smaller than the theory, possibly because of that SO_4^{2-} ions combined with Ca^{2+} ions in carriers to form a precipitate of $CaSO_4$, resulting in a decrease in effluent sulfate concentration. This phenomenon was demonstrated in a previous study [11]. Thus, this is also an advantage of sulfur-based carriers.

A further problem emerging from the sulfate generation was a pH decline. The denitrification performance is strongly affected by low pH value and the appropriate pH of 6.0 - 9.0 was determined for denitrified bacteria. The influent pH was 8.07 ± 0.34 and the effluent pH was 7.34 ± 0.13 for carrier C1 and 7.84 ± 0.06 for carrier C2. Although the sulfate content in the effluents is quite high, pH values were not strongly dropped. This is explained due to the high proportion (around 40%) of calcium carbonate in carriers, and $CaCO_3$ is also known as a pH buffer.

In addition, the nitrate removal rate obtained by applied sulfur-based carriers was obtained at concentration 0.34 ± 0.04 kg NO_3^--N/m^3/d. This removal rate is higher than that of other studies on autotrophic denitrification. For example, the nitrate removal rate was obtained at 0.24 kg NO_3^--N/m^3/d which using S^0 and $CaCO_3$ particles (size of 2 - 3 mm) [12], and the removal rate was reached at concentration only 0.08 - 0.28 kg NO_3^--N/m^3/d in the study which using S^0 and $CaCO_3$ particles (size of 3 - 15 mm) [13]. The higher nitrogen removal rate of this study compared to previous studies was shown in Table 3. Using small-size carriers (2 - 5 mm) presents several operational problems such as clogging, channeling, gas entrapment which reduce nitrogen mass transfer to bacteria in biofilms. The sulfur-based carriers made in this study can overcome those drawbacks and achieve at higher nitrogen removal efficiencies. Moreover, the capital cost for a wastewater treatment can be reduced, so these advantages are remarkable for practical applications.

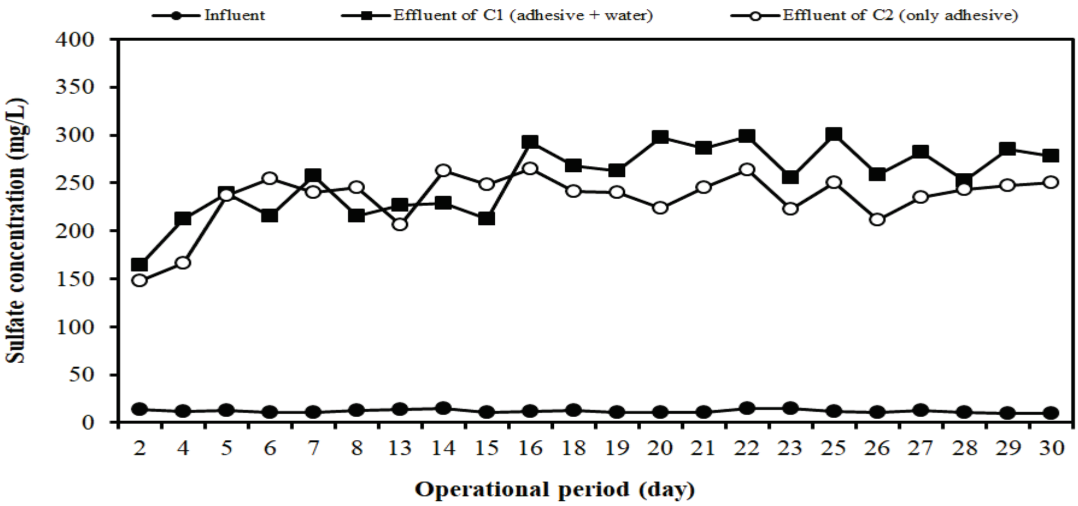

Fig. 5. Variation of sulfate concentration during the main operational phase

Table 3. Autotrophic denitrification in various studies

Ref.	Electron donor and alkalinity	Culture	Feed NO_3^--N (mg/L)	HRT (h)	Removal efficiency (%)	Removal rate (kg N/m^3/d)
[5]	S^0 particles (size of 3 - 5 mm) $NaHCO_3$	Conventional sludge	75	5	99	0.35
[13]	S^0 particles (size of 2 - 3 mm) $CaCO_3$ particles (size of 2 - 3 mm)	Enriched sludge	30	3	100	0.24
[14]	S^0 particles (size of 3.2 mm) $CaCO_3$ particles (size of 5.5 mm)	Enriched sludge	592	31	63	0.29
	S^0 fused with oyster shell (size of 11.3 mm)	Enriched sludge	592	31	81	0.37
	S^0 fused with powdered $CaCO_3$ (size of 10.7 mm)	Enriched sludge	592	31	34	0.15
This study	**Elemental sulfur-based carrier C1-C2 (size of 10 mm)**	**Enriched sludge**	**50**	**3**	**82-92**	**0.32 - 0.34**

4. Conclusion

The sulfur-driven autotrophic denitrification is a promising method to treat nitrogen compounds from water or wastewater which contain low C/N ratios, owing to less biomass production, no external organic carbon sources, and limiting green-house gas generation. Elemental sulfur-based carriers can be able to stimulate the occurrence of autotrophic denitrification. Adding water to the composition of the sulfur-based carrier can improve nitrate removal and reduce operational costs due to the increase in the porosity and pore size of carriers. Besides, the presence of $CaCO_3$ in carriers can reduce the actual ratio of generated sulfate to removed nitrate at the (SO_4^{2-}/NO_3^--N ratio) by $CaSO_4$ precipitation and limit the pH drop in bioreactors.

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